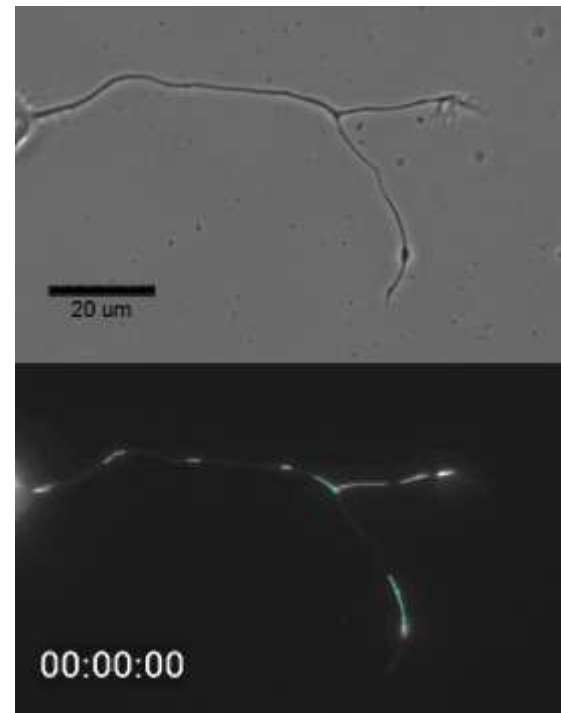




# Location and Organization of Features of the Mitochondrial Life Cycle in Neurons

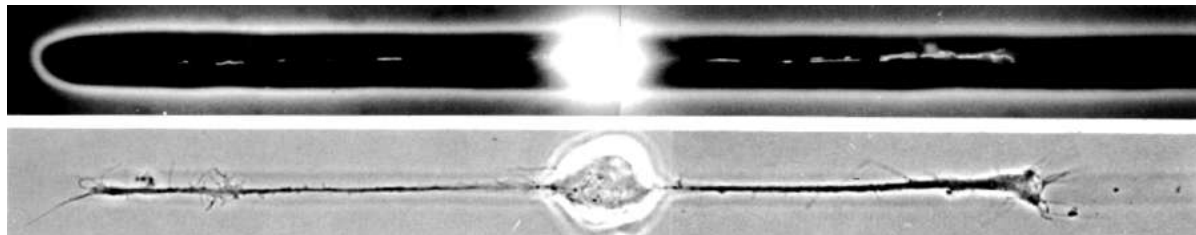
Peter J. Hollenbeck  
Dept of Biological Sciences  
Purdue University  
West Lafayette, IN

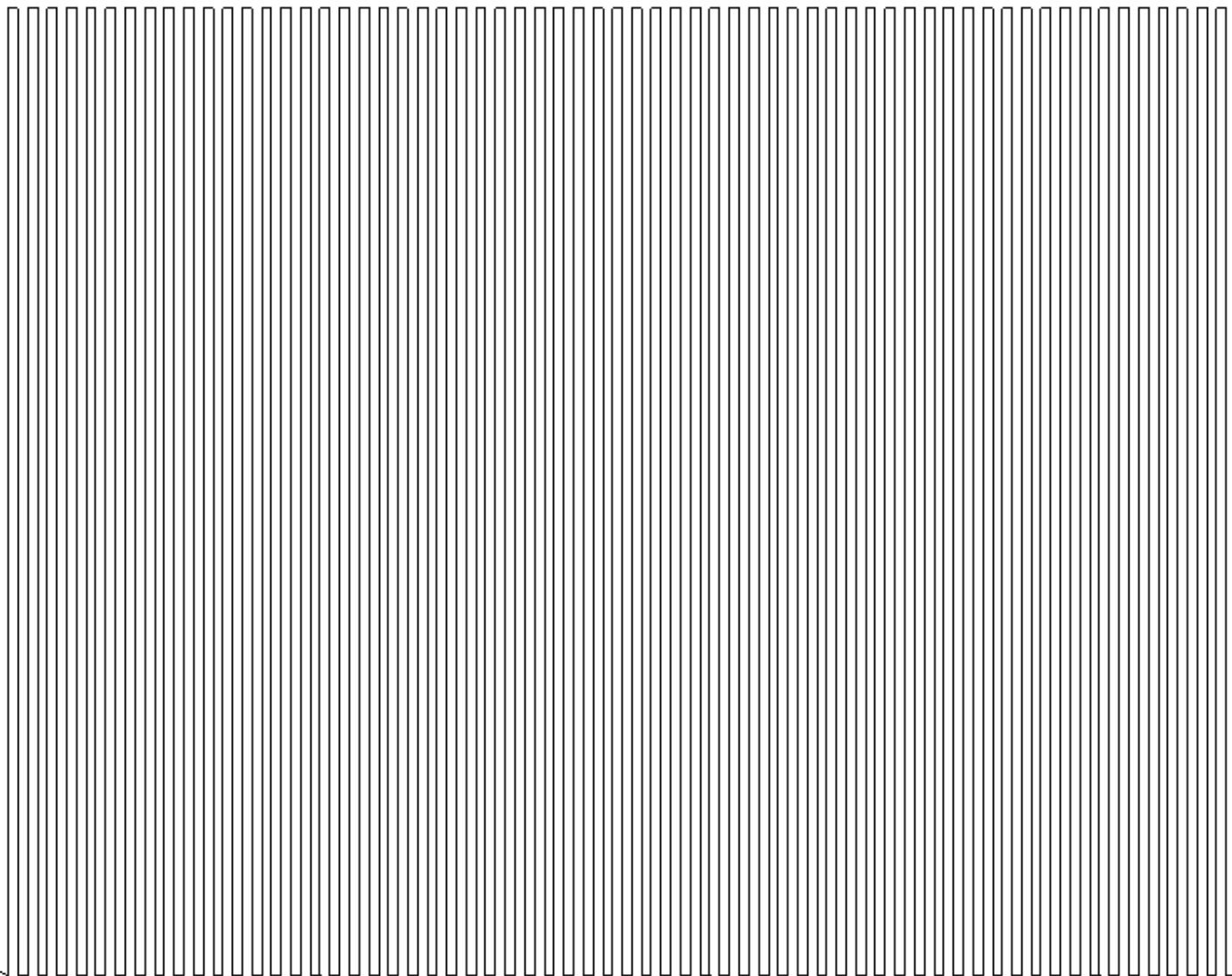
Workshop lecture 15  
ICTS-TIFR Advanced School on Axonal  
Transport & Neurodegenerative Disorders  
23 January 2013

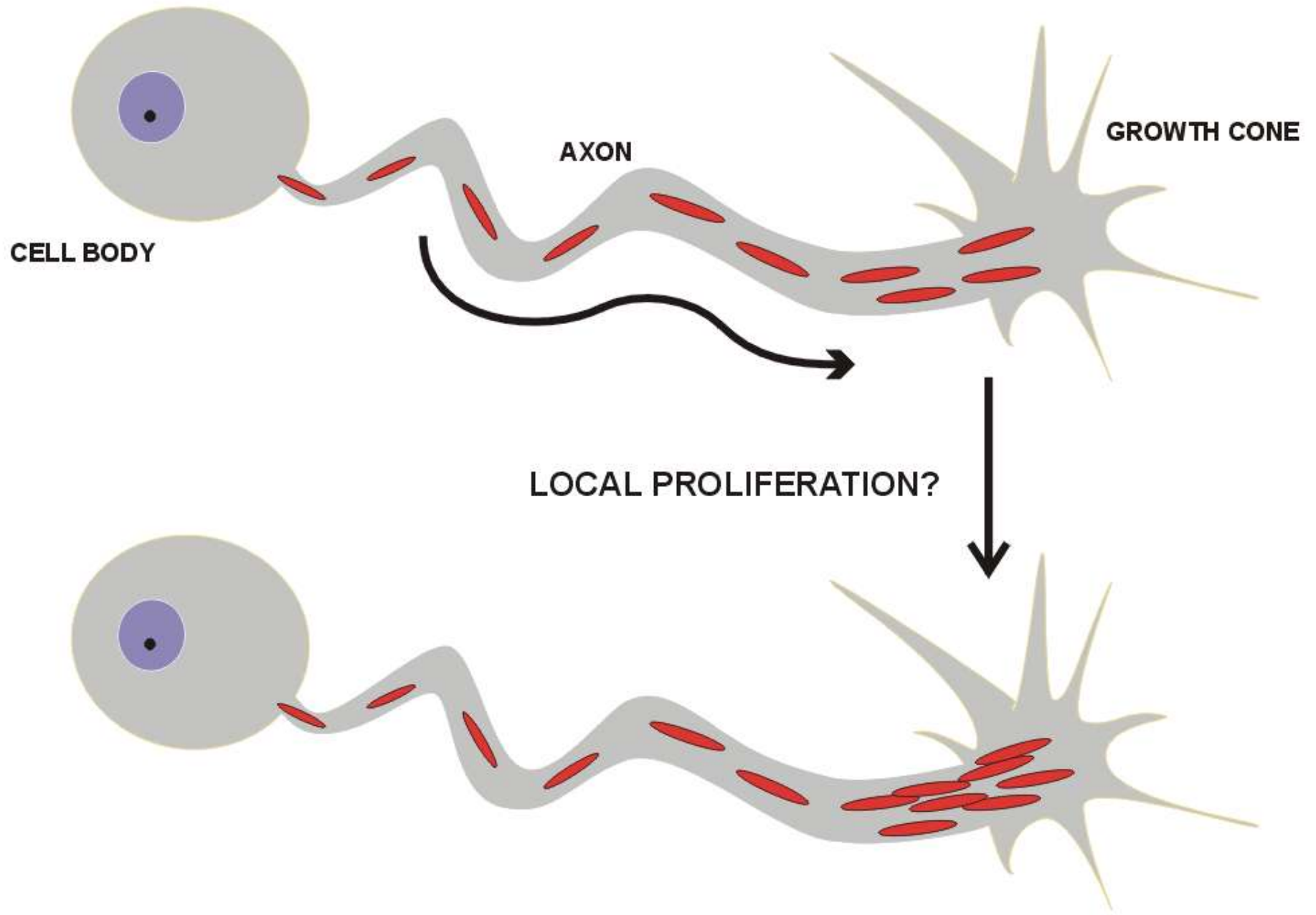


# Life cycle of mitochondria in the nervous system: how to supply the whole cell?

- (1) **Movement and distribution.** How do neurons get them to the right place at the right time?
- (2) **Metabolic activity.** Is their output modulated to meet the needs of time and place?
- (3) **Proliferation/biogenesis.** Can they divide and fuse in the axon, or are they just visitors?







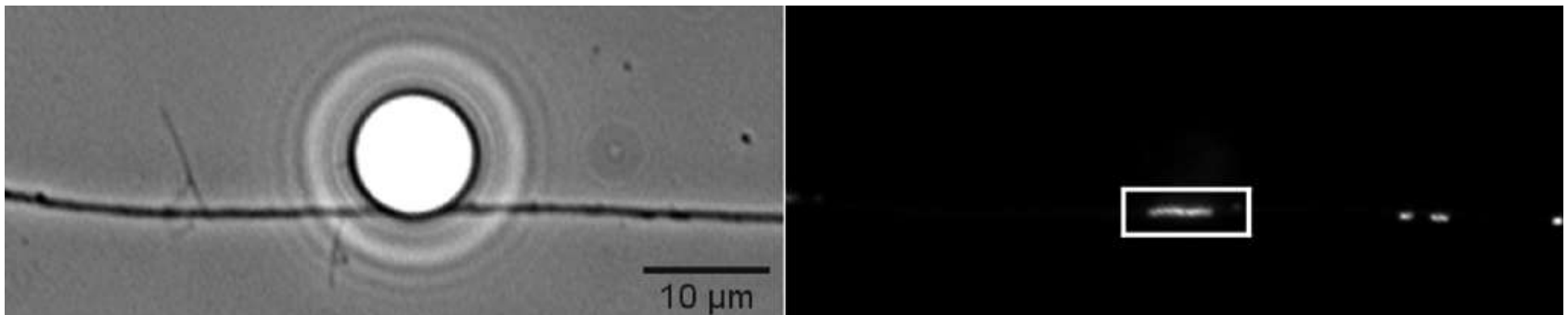
# Axonal transport of mitochondria: regulation by signaling

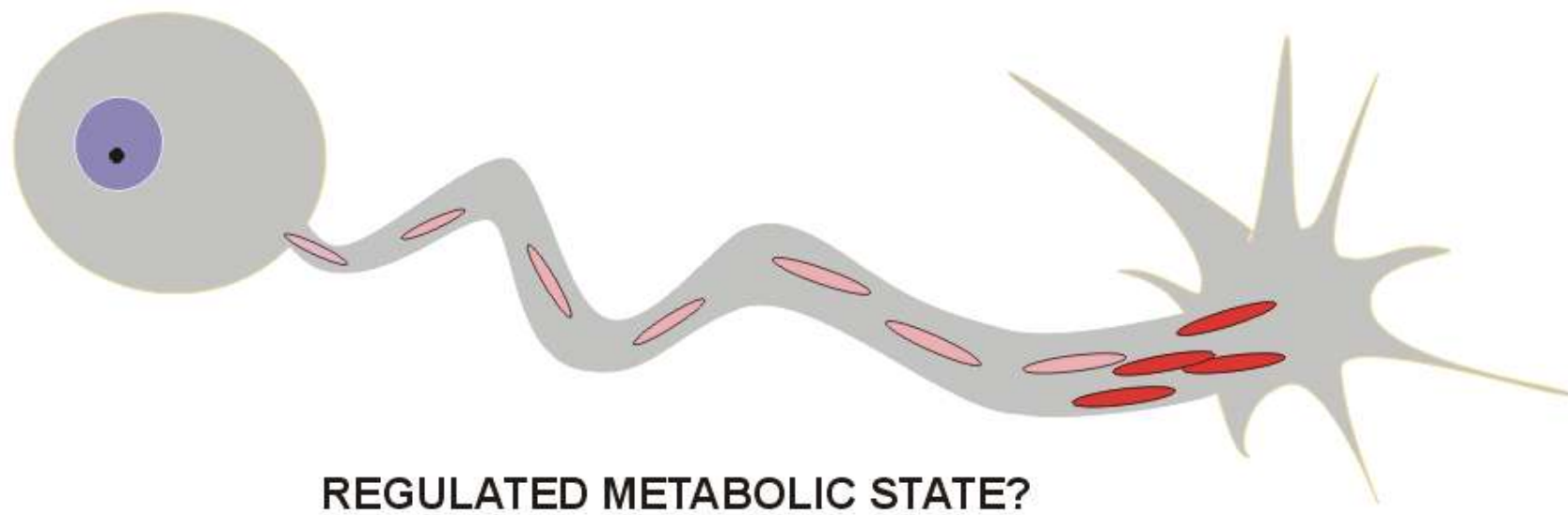
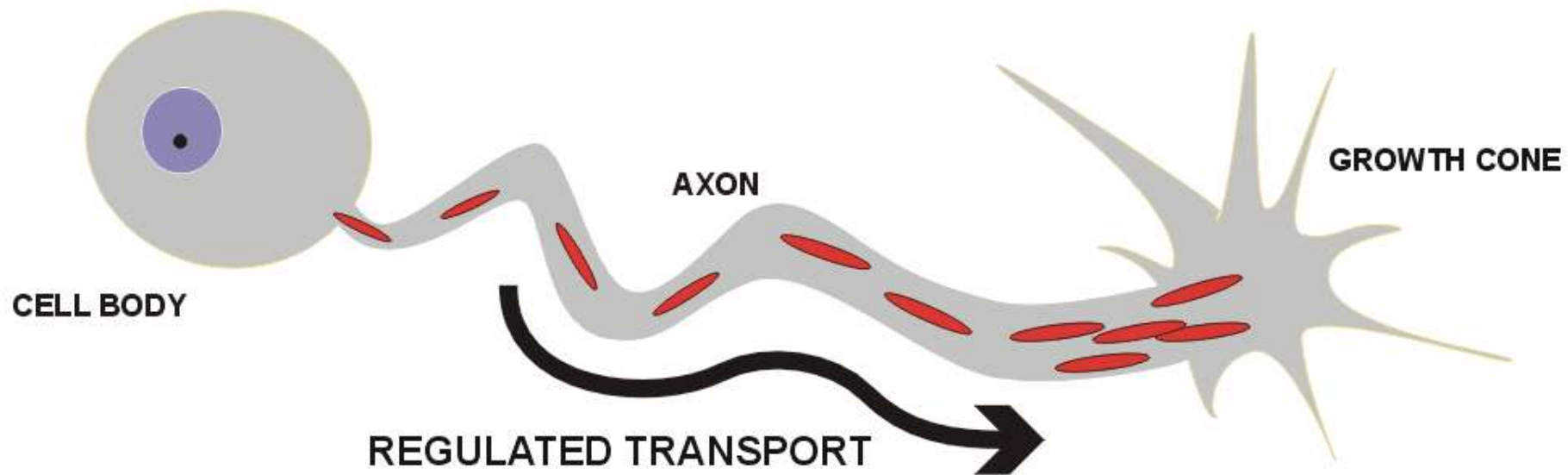
“Docking” of mitochondria can be induced by signaling from surface receptors:

Mitochondria halt at sites of NGF or sema 3A stimulation

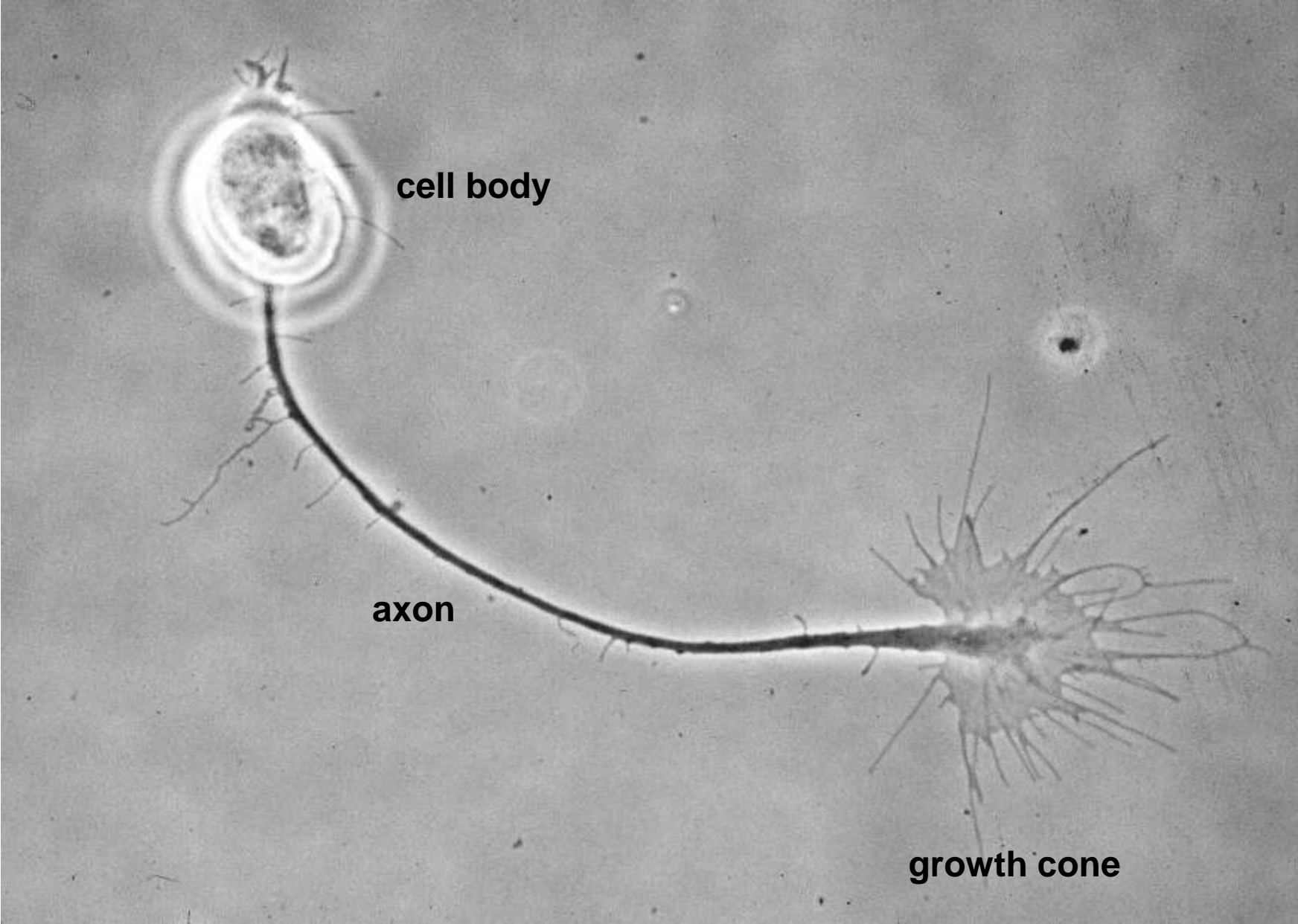
Docking requires the actin cytoskeleton & PI3K signaling

NGF-coated bead

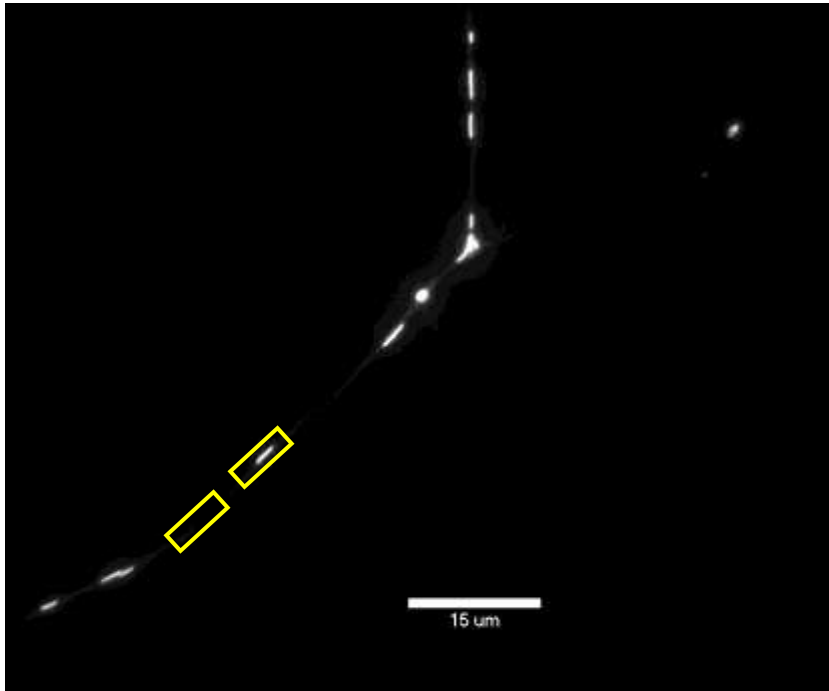




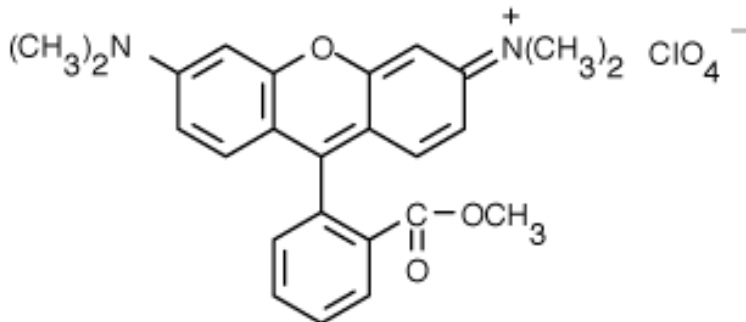
E10 chick sensory (DRG) neuron after 2 hrs in culture



# Mitochondrial metabolic activity: measuring $\Delta\Psi_m$



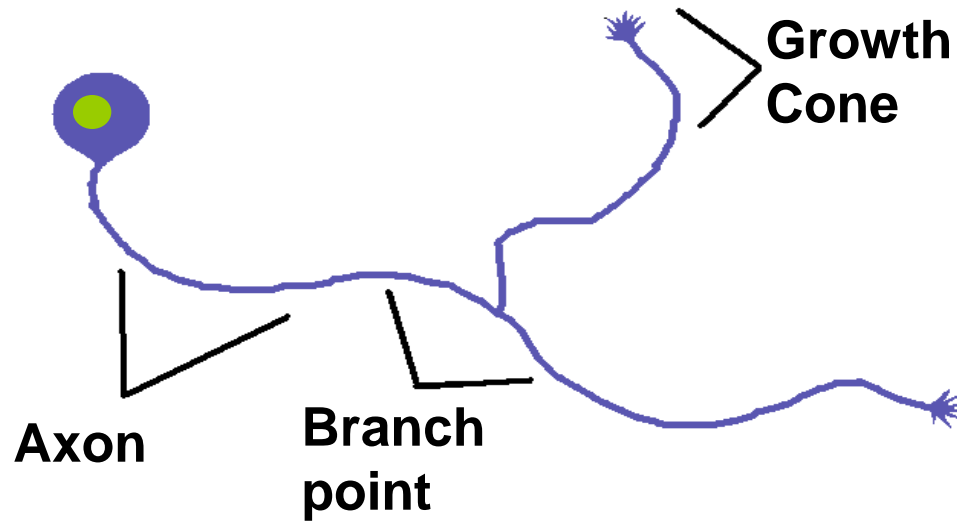
- Tetramethylrhodamine methyl ester (TMRM)
- Low toxicity, minimal interference with respiration
- Equilibrates quickly
- Ratio'd mito:cyto fluorescence intensity is proportional to  $\Delta\Psi_m$





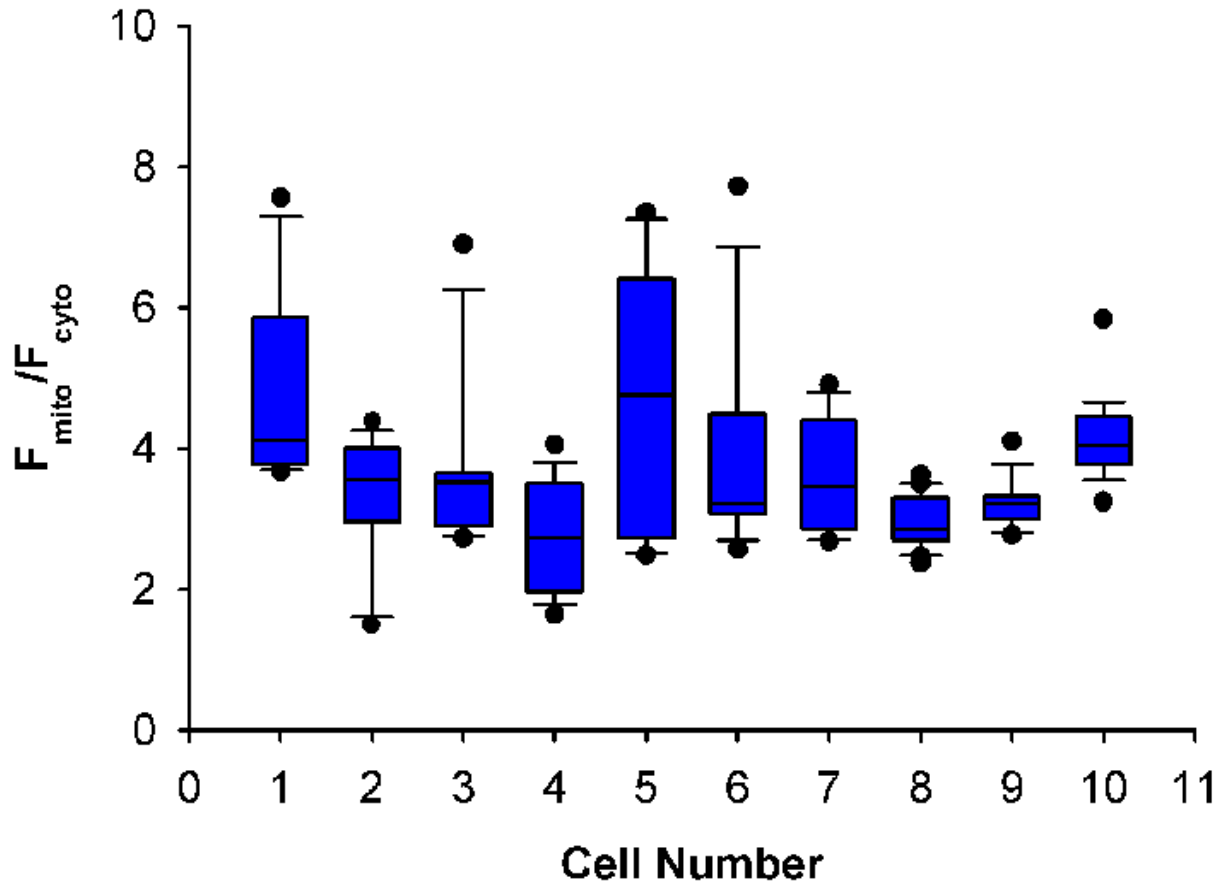
# Mitochondrial metabolic activity: variable?

Different states in **different regions**?



Membrane potential is similar in all regions of the axon, except **active growth cones**, where dye ratio is 27% higher.

$\Delta\Psi_m$  varies considerably among  
but not within cells



$\Delta\Psi_m$  varies considerably among  
but not within cells

Comparison of intercellular and intracellular  
variation in TMRM fluorescence ratio

<b>Mean <math>F_{\text{mito}}/F_{\text{cyto}}</math></b>	<b>11.03</b>	<b>Mean, all cells</b>
Intercellular variation	4.25	SD of means from each cell
Intracellular variation	1.77	Mean of SDs from each cell
n (neurons)	94	

$\Delta\Psi_m$  comparisons among mitochondrial populations  
using the TMRM  $F_{\text{mito}}/F_{\text{cyto}}$  ratio

No differences between:

Anterograde and stationary mitochondria in same axon

(n=40 neurons, p=0.4)

Retrograde and stationary mitochondria in same axon

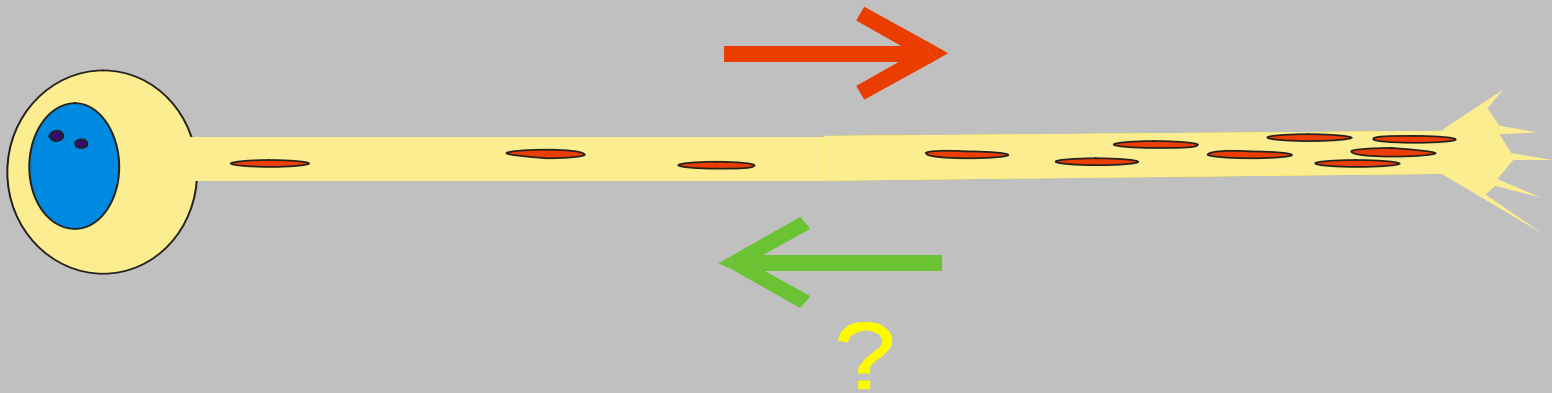
(n=28 neurons, p=0.66)

Anterograde and retrograde moving mitochondria

(p=0.43)

# Mitochondrial metabolic activity: variable?

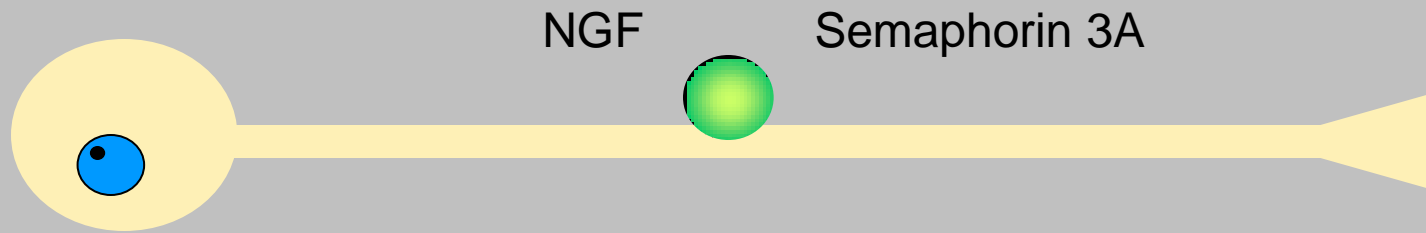
Different states for mitochondria moving in  
**different directions?**



NO – we **cannot** distinguish differences in membrane potential among anterograde, retrograde, and stationary mitochondria.

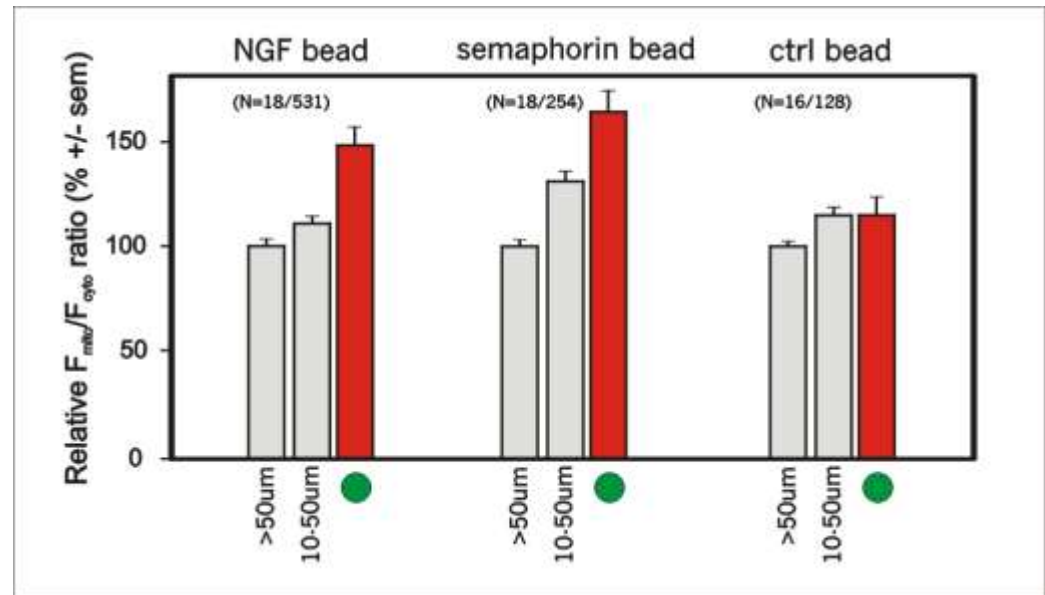
# Mitochondrial metabolic activity: variable?

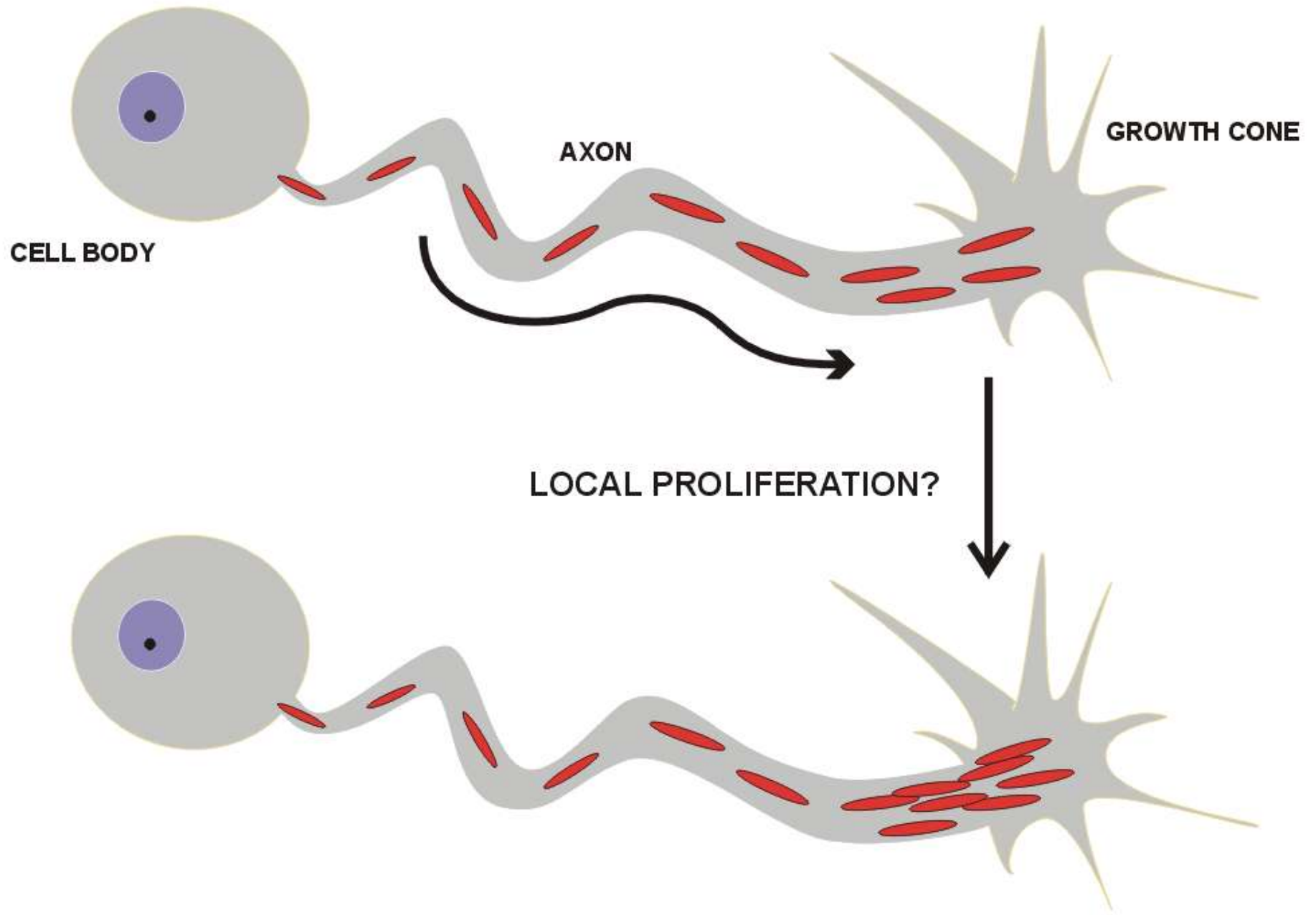
Regulated by **cell signaling**?



$F_{\text{mito}}/F_{\text{cyto}}$  **increased 50%** in immediate vicinity of NGF or semaphorin stimulation.

Also **decreased 75%** when RTKs were broadly inhibited (tyrphostin AG 879)





**Wait:** exactly what are the measures of “mitochondrial proliferation” or “biogenesis”?

Mitochondria are normally a sort of **syncytium** (even in the somatodendritic compartment).

But their **transport** along the axon gives individual mitochondria a **longer independent identity** than almost anywhere else, we think (kiss and run?).

We have tried to observe and **quantify**:

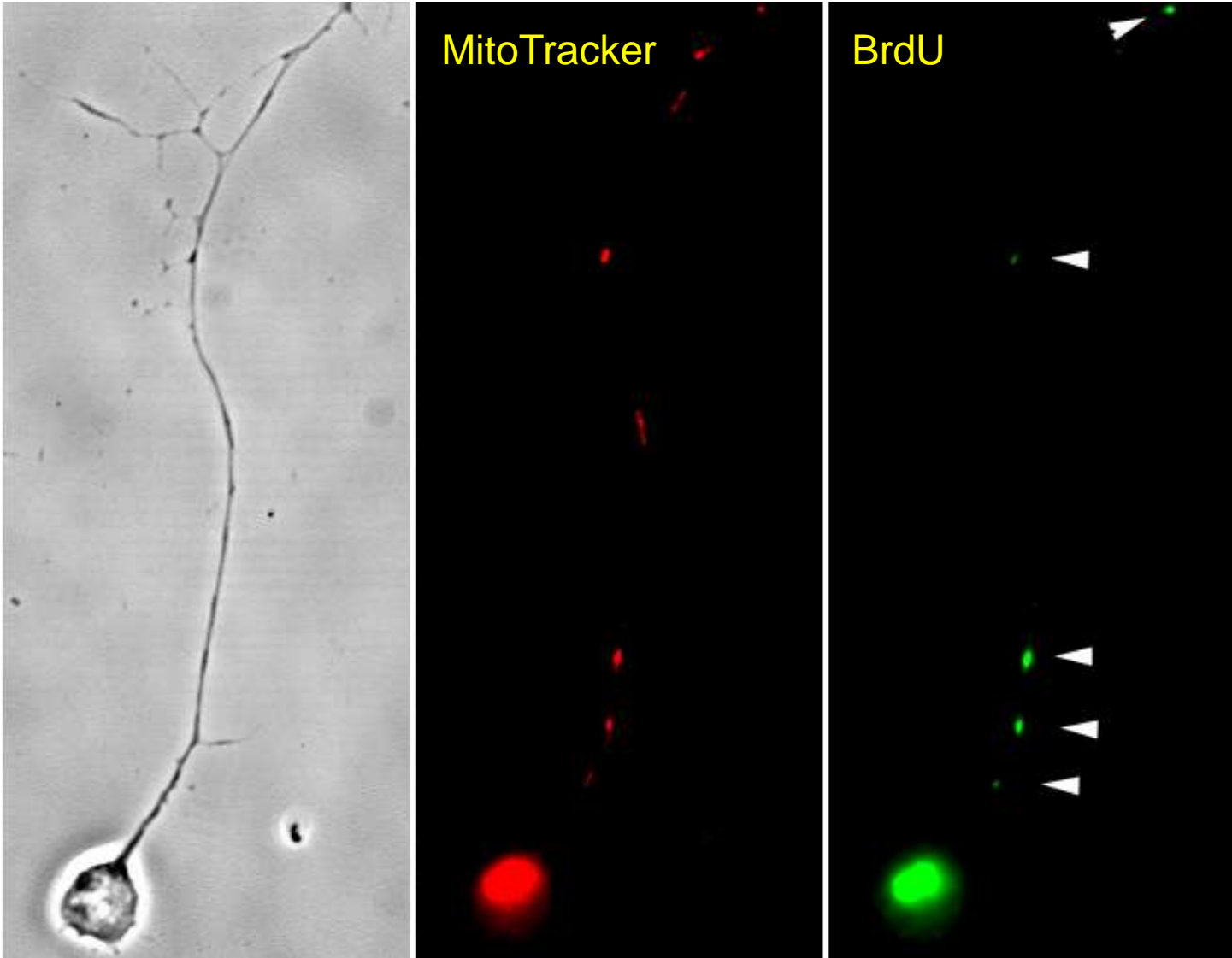
mtDNA synthesis

Mitochondrial fission

Mitochondrial fusion

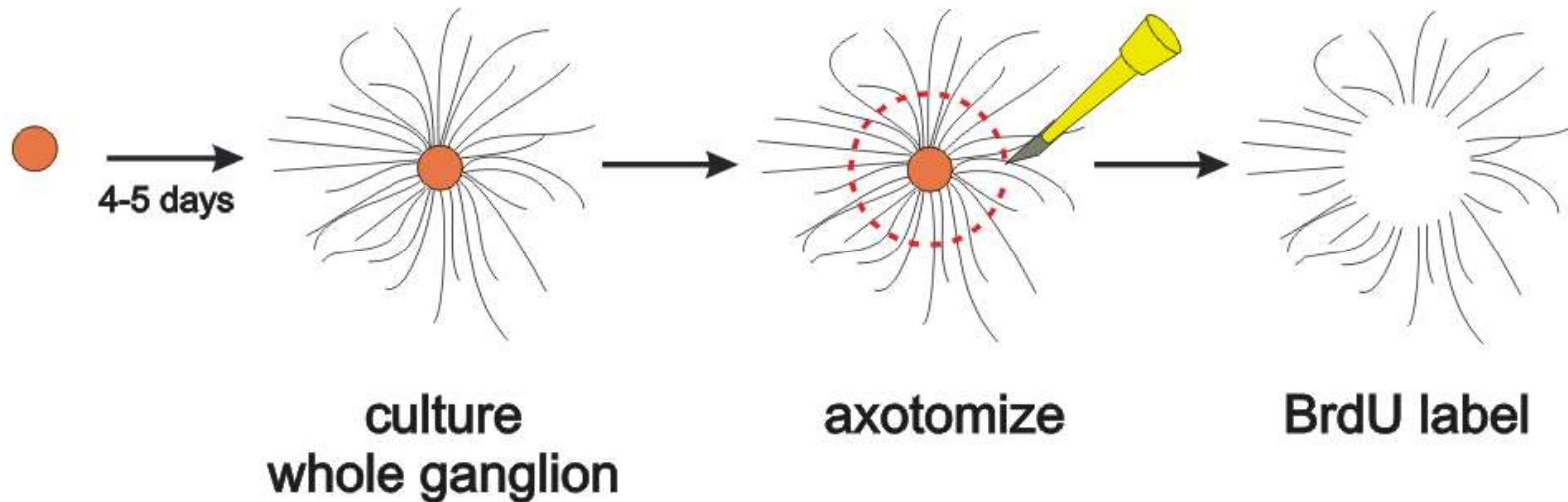


# Colocalization of BrdU with MitoTracker

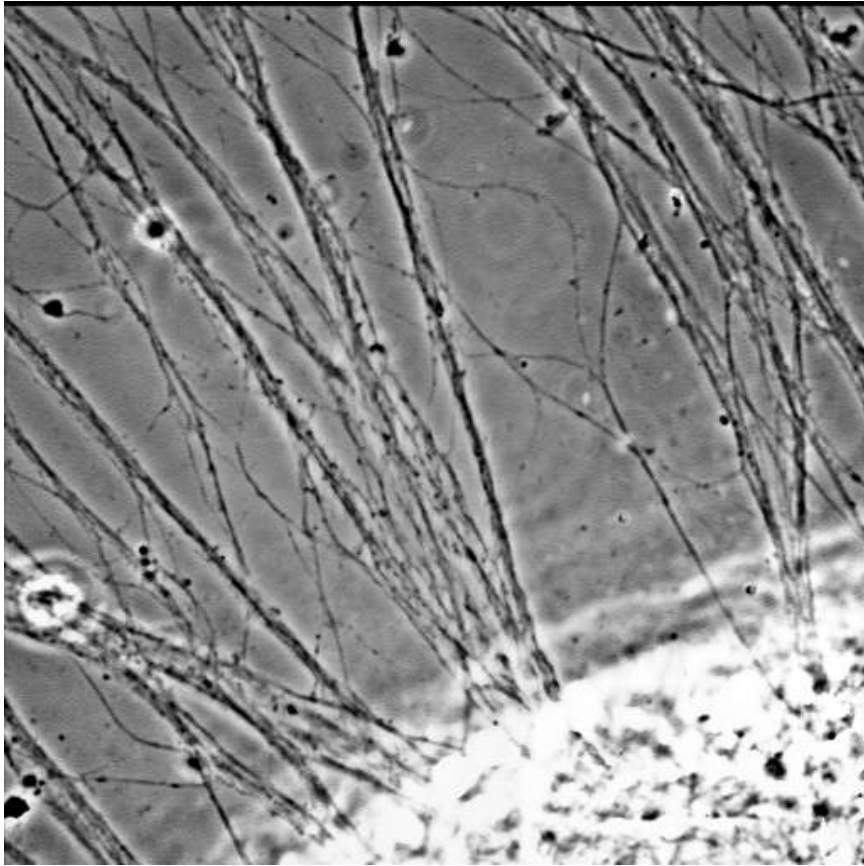


# Mitochondrial biogenesis: can it occur in the axon?

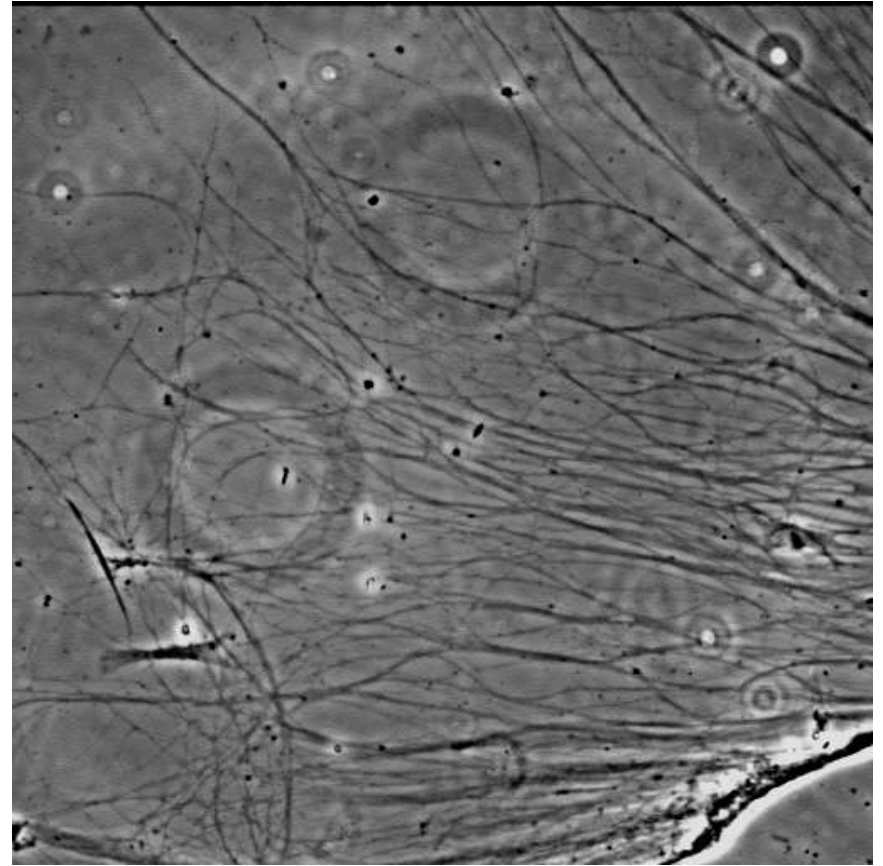
mtDNA replication



# Sympathetic ganglia

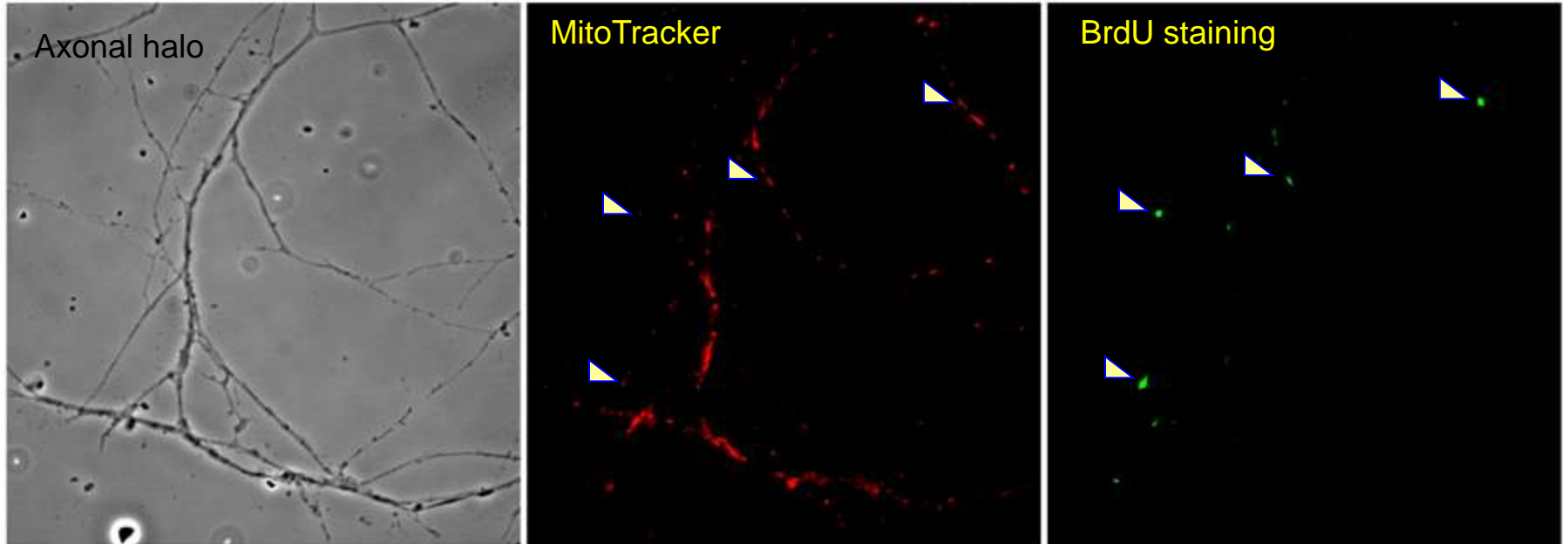


Intact



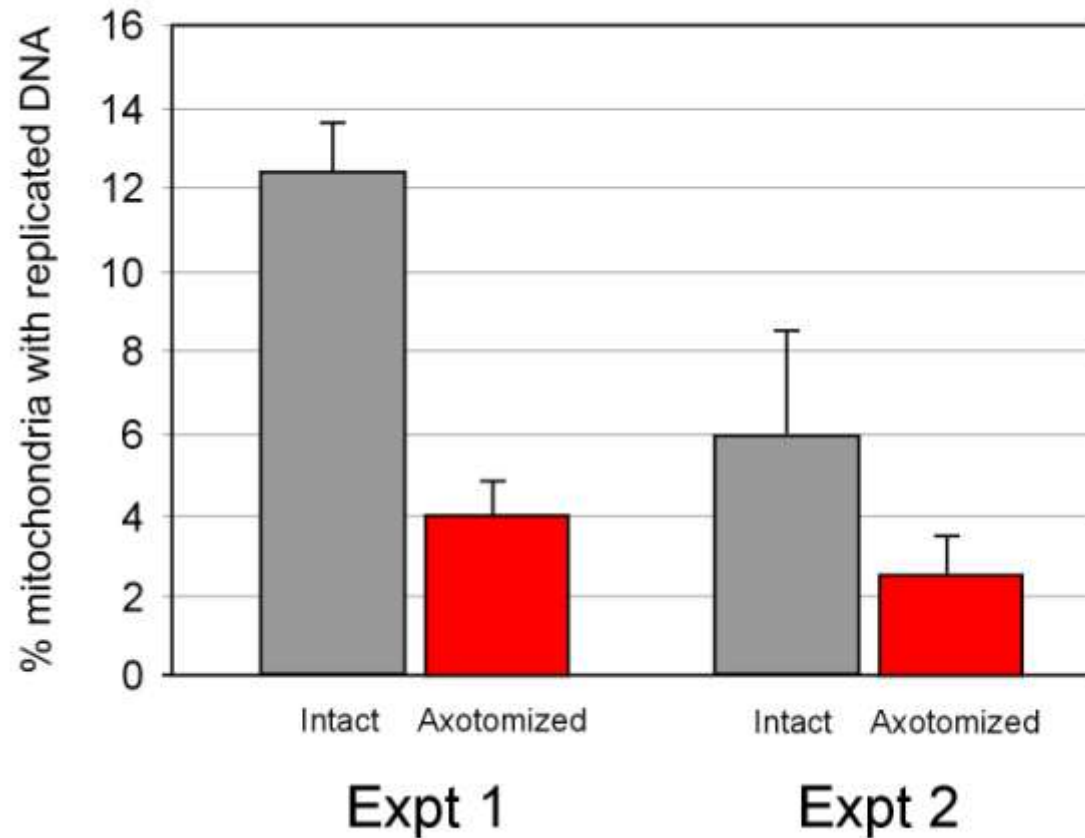
Axotomized  
(cell body mass removed)

mtDNA replication can occur in axons completely separated from their cell bodies



mtDNA replication **can occur** in axons in complete **absence of cell bodies**. How frequent is it?

# Axonal mitochondrial DNA replication with or without connection to the cell body



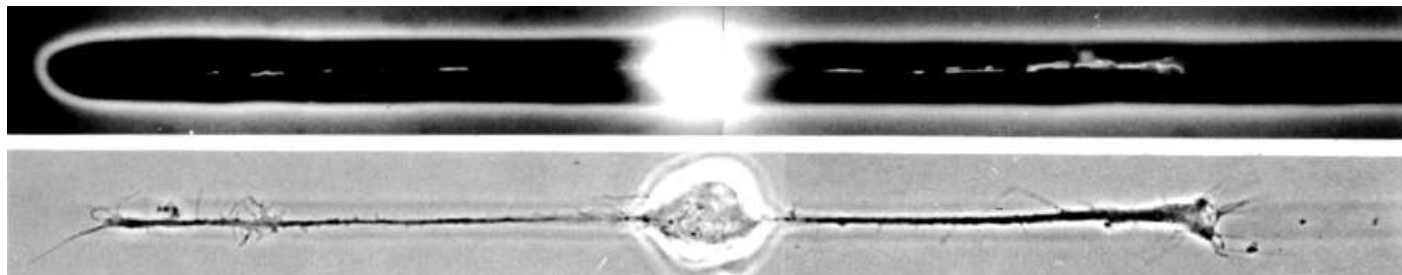
~1/3 as much mtDNA synthesis in axotomized cultures

# Mitochondrial biogenesis: can it occur in the axon?

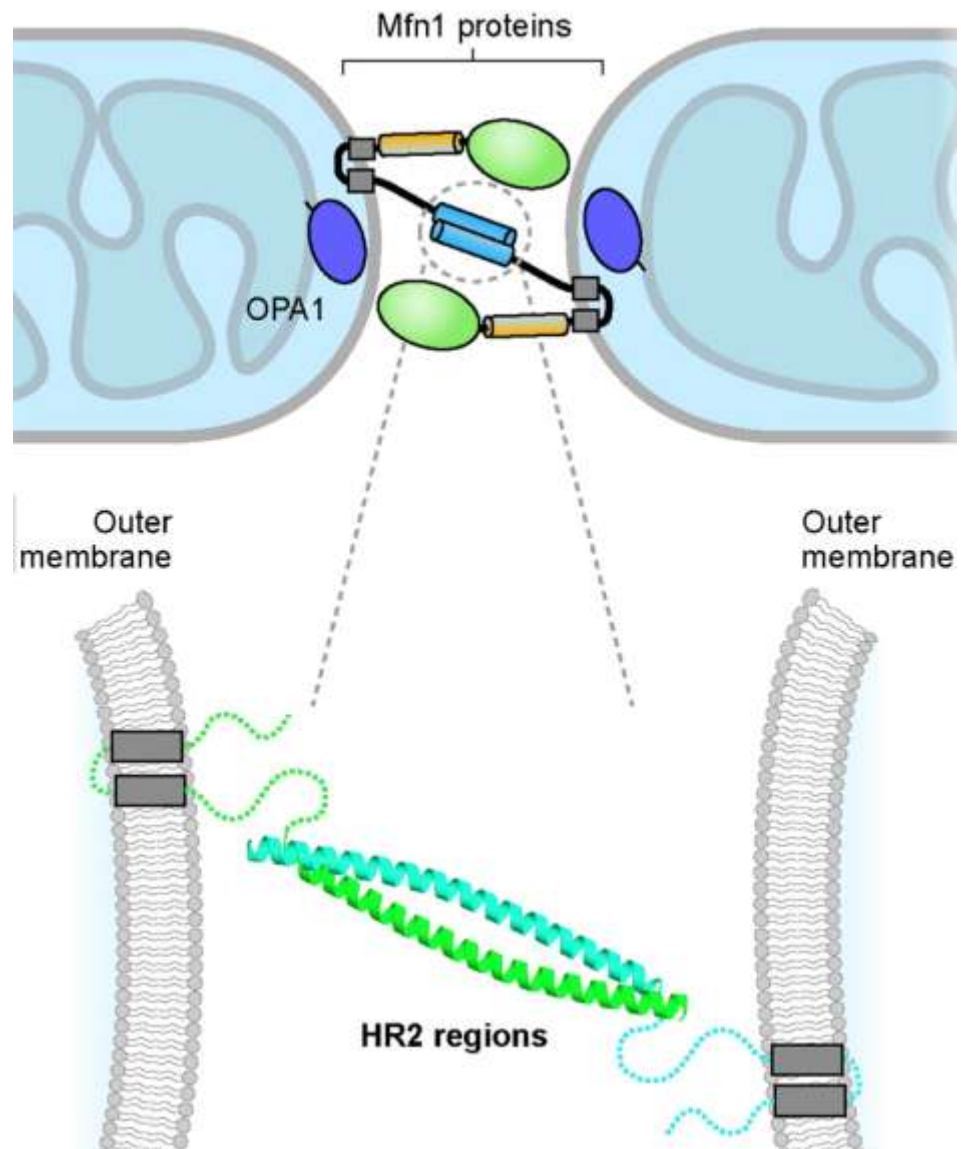
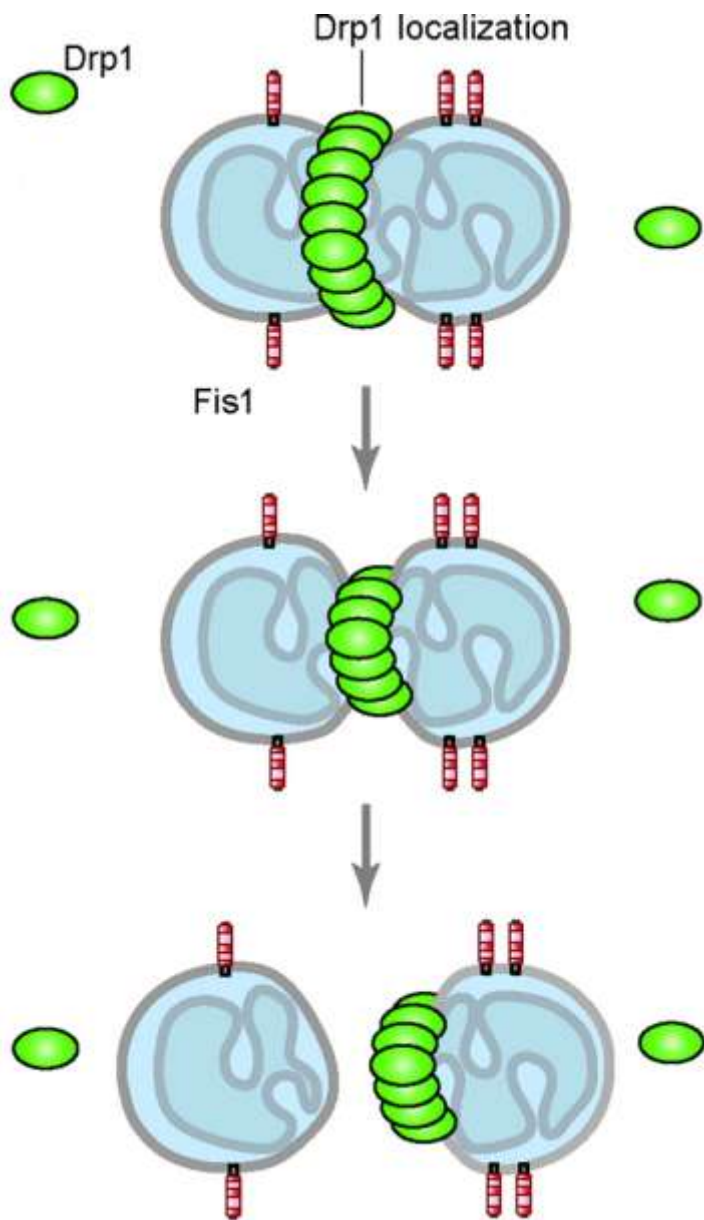
Do axonal mitochondria undergo fission and fusion, or are these processes restricted to the cell body?

Following mitochondria in live axons has not allowed unambiguous detection of fission-fusion events (yet).

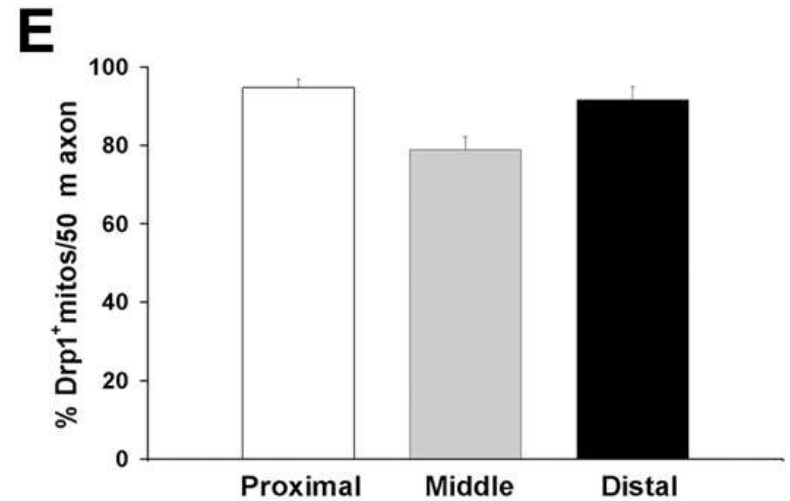
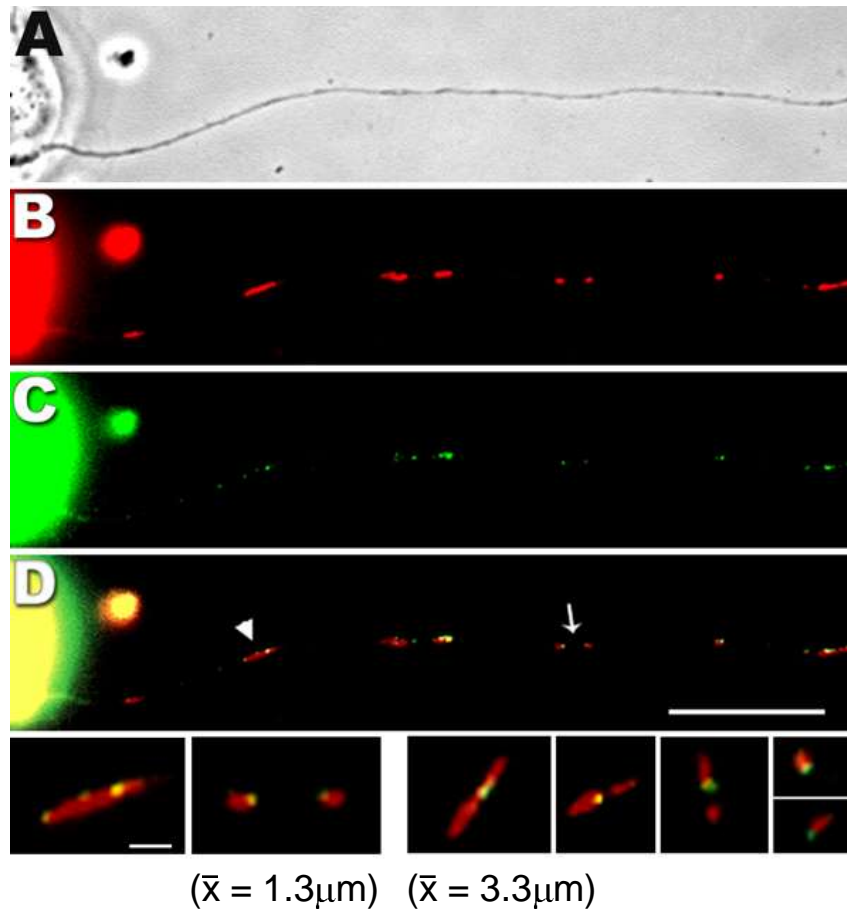
Can other approaches yield indirect evidence for fission-fusion?



# Mitochondrial fission and fusion – two key proteins



The fission protein Drp1 is found on most mitochondria in the axon

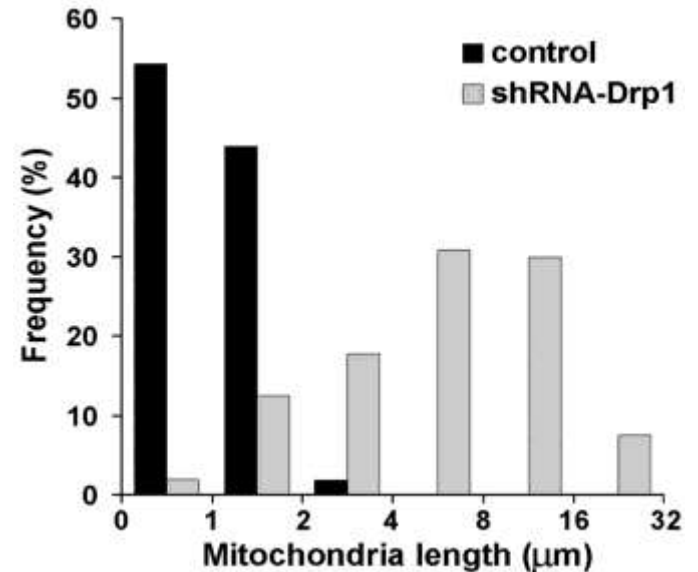
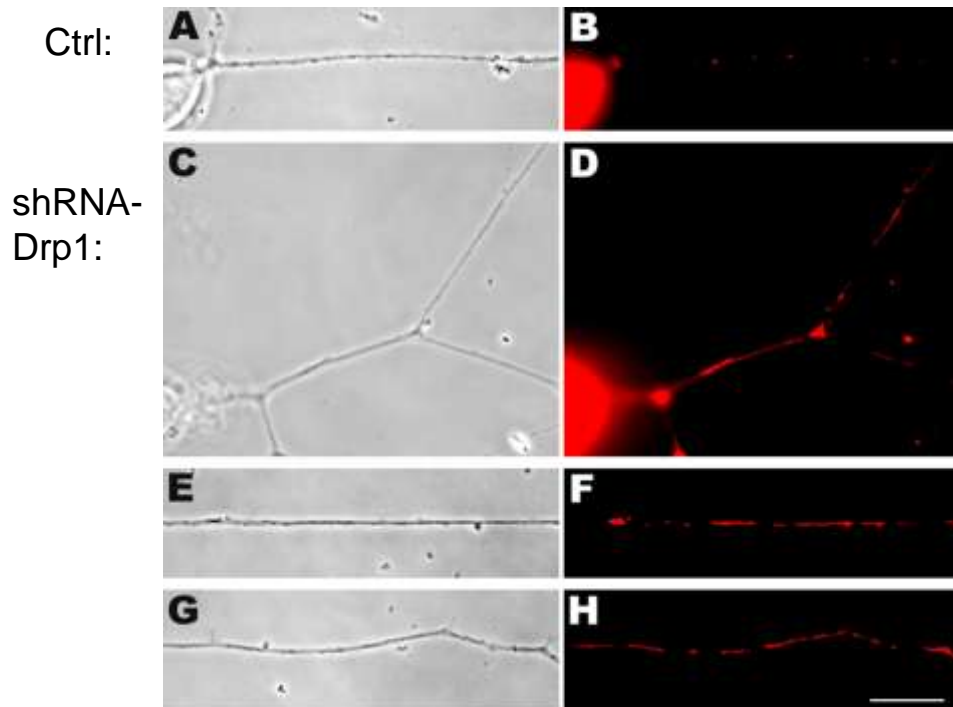


Drp1 disposition on short vs long mitochondria is consistent with the occurrence of fission in the axon



# Mitochondrial biogenesis: can it occur in the axon?

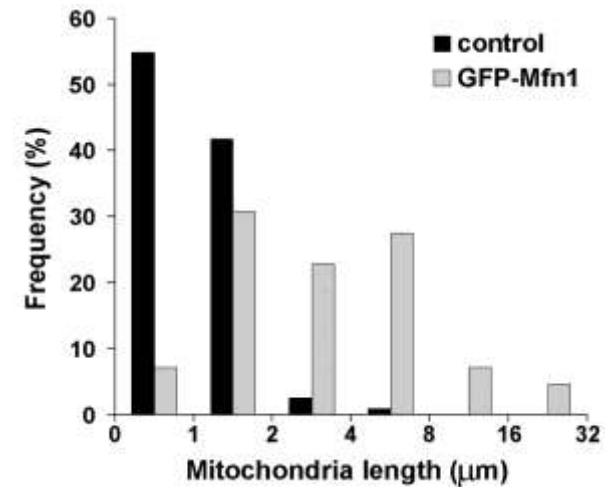
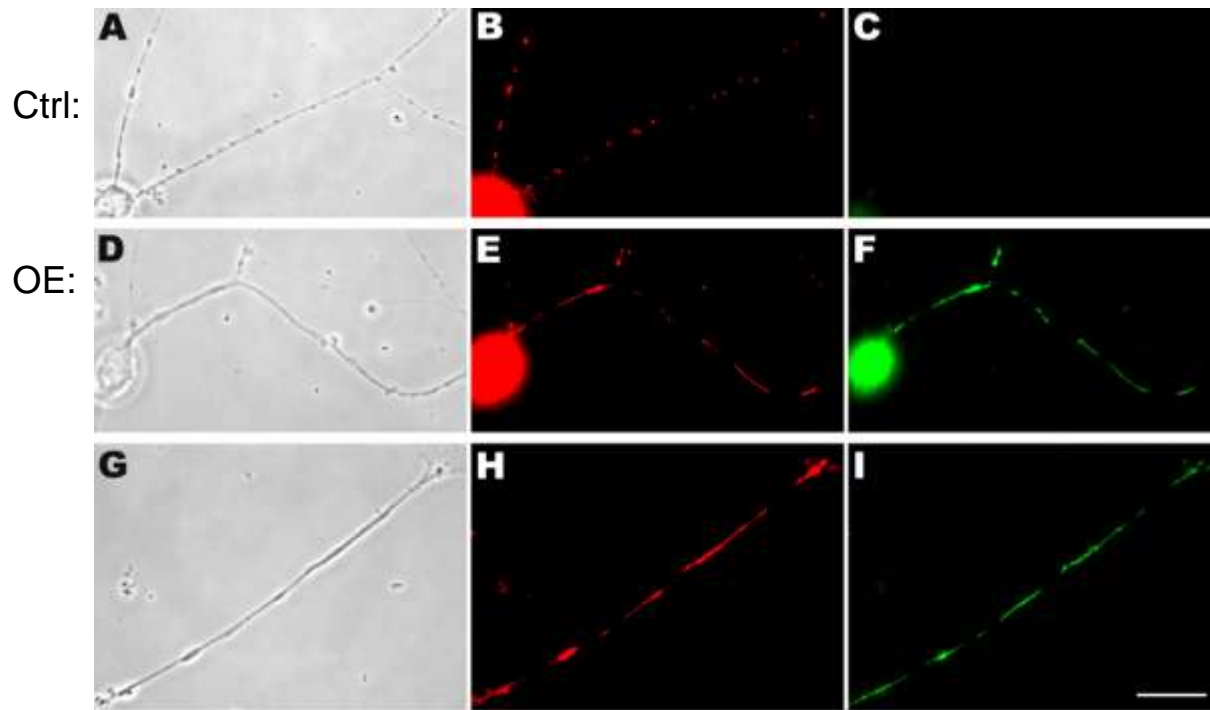
Knock-down of Drp1: suppress mitochondrial fission:



RNAi knock-down of the fission protein Drp1 results in much longer axonal mitochondria

# Mitochondrial biogenesis: can it occur in the axon?

OE of Mfn1: stimulate mitochondrial fusion:



Over-expression of fusion protein mitofusin (Mfn1) also results in much **longer axonal mitochondria**

# Mitochondrial life cycle: what's going on in the axon?

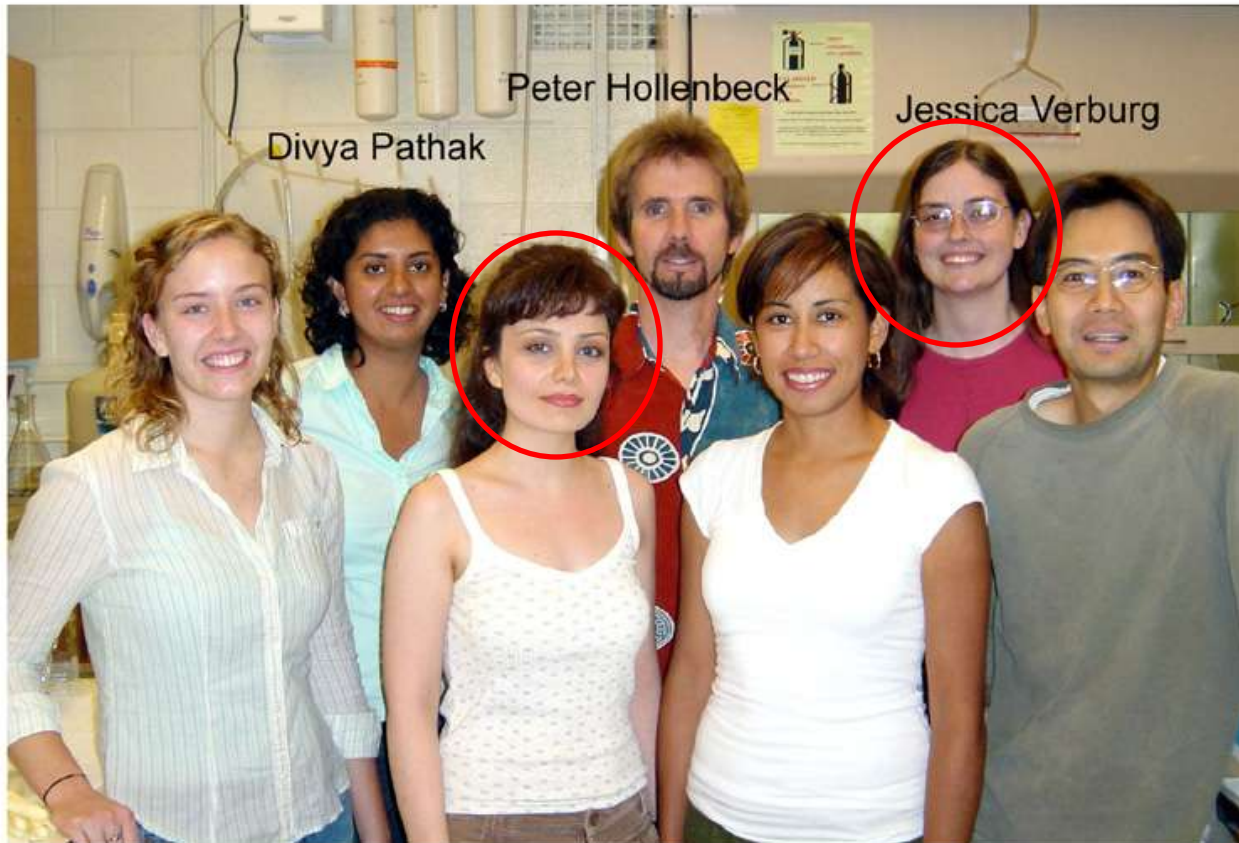
**Motility and docking** can be controlled by cell signaling – stay tuned.

Membrane potential can **vary regionally** (e.g., growth cones) but probably does **not** vary between anterograde, retrograde and stationary mitochondria.

Membrane potential **responds** to attractive and repulsive signals, and to RTK signaling.

Mitochondria can **replicate their DNA** within the axon, and this could represent a large fraction of the total neuronal mtDNA synthesis.

**Fission and fusion** almost certainly occur in the axon, including very far from the cell body. Stay tuned for direct evidence.



Valerie Turner

Mandana Amiri

Cecilia Martinez

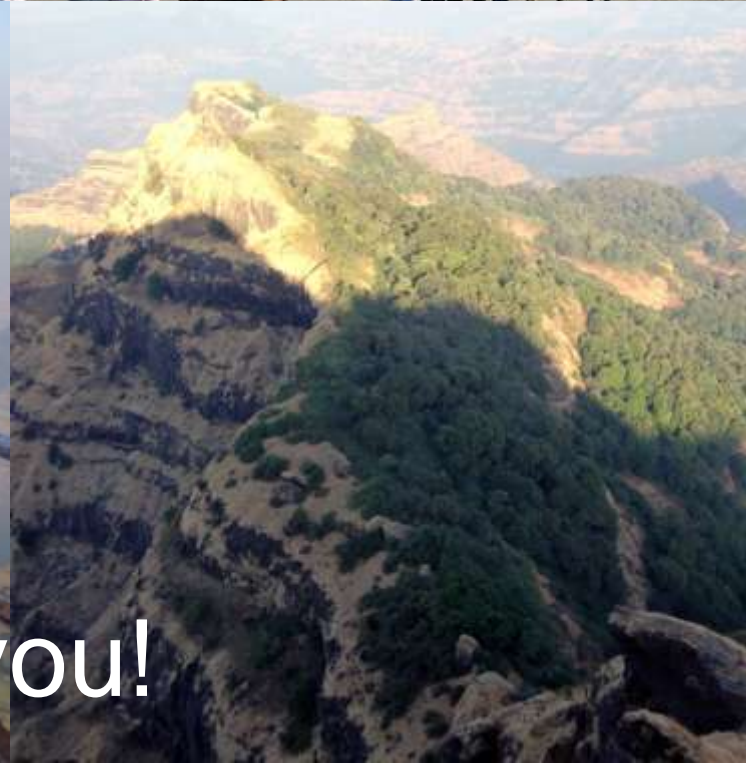
Yujiro Shidara

Divya Pathak

Peter Hollenbeck

Jessica Verburg

*Funding from NINDS*



Thank you!